Promega

Product Application

# Automated Purification of DNA from Mold Spores

*Purify DNA from Aspergillus spores using the Maxwell® RSC PureFood GMO and Authentication Kit on a Maxwell® RSC Instrument.*

**Kit:** Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)

**Analyses:**

- UV absorbance
- Dye-based quantitation
- qPCR
- TapeStation Analysis

**Sample Type(s):** Mold spores (*Aspergillus*)

**Input:** ≤ 200mg

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM473, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

**Materials Required:**

- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Maxwell® RSC Instrument or Maxwell® RSC 48 Instrument
- Liquid nitrogen, mortar, and pestle *OR*
- Bead beating supplies
  - Lysing Matrix A – MP Biomedicals, Cat.# 6910050
  - Digital Vortex Genie II – Scientific Industries or similar
  - Horizontal Vortex Adaptor for 1.5-2.0ml tubes – Qiagen, Cat.# 13000-V1 or similar
- Heat block set to 65°C, preferably with shaking (e.g. Thermomixer R)

**Protocol**:

1. If using a mortar and pestle for sample disruption
   a. Transfer tissue to a mortar pre-cooled with liquid nitrogen.
   b. Grind for several minutes using a pre-cooled pestle.
   c. Transfer ≤ 200mg of ground tissue to a 1.5ml tube.
   d. Move tubes to room temperature and add 500µl of CTAB, 20µl of RNase A, and 40µl of Proteinase K.
2. If using bead beating for sample disruption
   a. Transfer ≤ 200mg of tissue to a Lysing Matrix A tube.
   b. Add 500µl of CTAB.
   c. Secure sample tubes on a digital vortex outfitted with a horizontal tube adaptor. Vortexes may require balancing of tubes and/or limited tube numbers to maintain vortex speed.
   d. Vortex for 10 minutes at 2600-3000rpm.

   e. Remove tubes from the vortex and add 20µl of RNase A and 40µl of Proteinase K.

3. Place samples in a heat block at 65°C for 1.5 – 2 hours with shaking at 600rpm.
4. During incubation, prepare RSC cartridges as described in the Maxwell® RSC PureFood GMO and Authentication Kit Technical Manual.
   a. Add 300µl of Lysis Buffer to Well #1.
   b. Place a plunger in Well #8.
   c. Add 100µl of Elution Buffer to each elution tube.
5. After incubation, centrifuge samples for 10 minutes at ≥16,000 × g.
6. Transfer entire clear supernatant (up to 500µl) to Well #1 of the reagent cartridge. Avoid pipetting any solid material.
7. Place the prepared cartridges in the Maxwell® RSC Instrument and run the Maxwell® RSC PureFood GMO and Authentication Kit method.

**Results:**

DNA was purified from *Aspergillus* spores using the Maxwell® RSC PureFood GMO and Authentication Kit as described above. Purified DNA typically showed low absorbance ratios (Figure 1), but all samples could be amplified by qPCR (Figure 2).

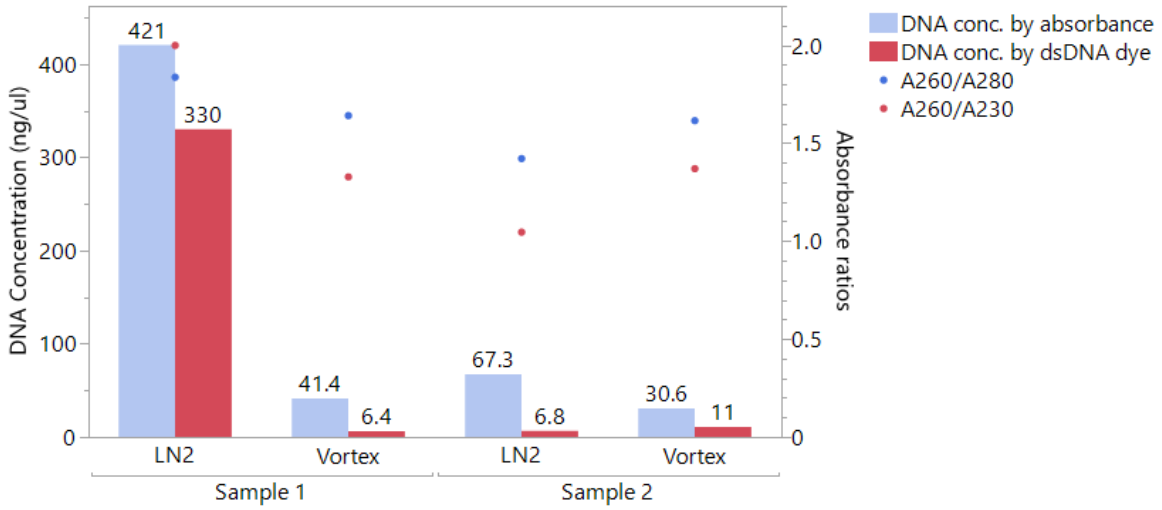


**Figure 1. Concentration and absorbance ratios of DNA purified from *Aspergillus* spores using the Maxwell® RSC PureFood GMO and Authentication Kit.** DNA was purified from two distinct samples of *Aspergillus* spores using either disruption by grinding under liquid nitrogen (LN2) or vortexing for 10 minutes in Lysing Matrix A tubes as described in the protocol above. DNA concentration and A260/A280 and A260/A230 absorbance ratios were measured by absorbance using a NanoDrop™ ONE Spectrophotometer. DNA concentration was also measured using QuantiFluor® ONE dsDNA System (Cat.# E4871) on a Quantus™ Fluorometer (Cat.# E6150). Mean of n=2 shown for Sample 2, LN2; N=1 for all other samples.

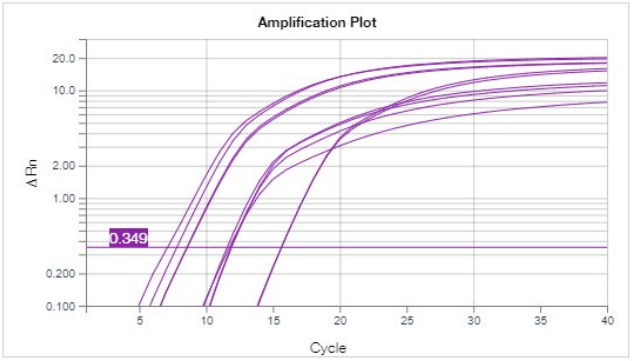


**Figure 2. DNA amplification.** DNA eluates shown in Figure 1 were amplified in duplicate with GoTaq® qPCR Master Mix (Cat.# A6001) and universal fungal 18S rRNA gene primers[1] on a QuantStudio™ 6 Pro Real-Time PCR Instrument (Applied Biosystems™). All samples were amplified undiluted (2µl in a 20µl reaction) except "Sample 1 LN2", which was diluted 1/10 in Nuclease-Free Water due to its high concentration. Semi-log plot is shown.

**References:**

1. Embong, Z, Hitam, W., Yean, C., et. al. (2008) Specific detection of fungal pathogens by 18S rRNA gene PCR in microbial keratitis. BMC Opthalmol. 8:7.